

Constrained geometric dynamics of the Fenna-Matthews-Olson complex

The role of correlated motion in reducing uncertainty in excitation energy transfer

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Abstract The trimeric Fenna-Matthews-Olson (FMO) complex of green sulphur bacteria is a well-studied example of a photosynthetic pigment-protein complex, in which the electronic properties of the pigments are modified by the protein environment to promote efficient excitonic energy transfer (EET) from antenna complexes to the reaction centres. By a range of simulation methods, many of the electronic properties of the FMO complex can be extracted from knowledge of the static

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crystal structure. However, the recent observation and analysis of long-lasting quantum dynamics in the FMO complex point to protein dynamics as a key factor in protecting and generating quantum coherence under laboratory conditions. While fast inter- and intra- molecular vibrations have been investigated extensively, the slow, conformational dynamics which effectively determine the optical inhomogeneous broadening of experimental ensembles has received less attention. The following study employs constrained geometric dynamics to study the flexibility in the protein network by efficiently generating the accessible conformational states from the published crystal structure. Statistical and principle component analysis reveal highly correlated low frequency motions between functionally relevant elements, including strong correlations between pigments that are excitonically coupled. Our analysis reveals a hierarchy of structural interactions which enforce these correlated motions, from the level of monomer-monomer interfaces right down to the α -helices, β -sheets and pigments. In addition to inducing strong spatial correlations across the conformational ensemble, we find that the overall rigidity of the FMO complex is exceptionally high. We suggest that these observations support the idea of highly correlated inhomogeneous disorder of the electronic excited states, which is further supported by the remarkably low variance (typically $<5\%$) of the excitonic couplings of the conformational ensemble.

Keywords Trickle Down Structural Organisation · Clam Shell · EET · Correlated Motion · FRODA

1 Introduction

The early stages of photosynthesis involve ultrafast photophysics, which facilitate the capture, transport, and, ultimately, utilisation of photonic energy. Thereafter, photosynthetic organisms are able to initiate a much slower sequence of complex chemical events that are necessary for life. These initial stages, known as the light reactions, employ optically active molecules (pigments) tuned to the organism's photonic environment. Light-harvesting proteins have been developed in Nature for the purpose of co-ordinating the pigments and other cofactors. They are employed for a variety of different functions, such as energy transporting antennas, charge separation centres, photoprotection mechanisms, and so forth (Blankenship, 2002; Van Amerongen et al, 2000). The versatility of the

pigment-protein organisational paradigm has enabled photosynthetic organisms to colonise a diverse array of environments, and there is currently a great interest in learning how the reproducible control and efficiency of nanoscale energy and charge processes in natural pigment-protein complexes (PPCs) is realised (Scholes et al, 2011).

Part of the motivation for this is the rapid advance of nanoscale materials engineering in the field of solar light harvesting, particularly in organic optoelectronics, where biological solutions to problems of efficient and directed excitation energy transfer (EET) and stable, long-range charge separation could suggest new ways to break these otherwise limiting constraints in artificial devices (Scholes et al, 2011; Dimitrov and Durrant, 2013). Moreover, the scope of what may be learned from PPCs has recently been expanded due to the unexpected observation of room temperature quantum mechanical dynamics in a range of PPCs extracted from bacteria, plants and algae (Lee et al, 2007; Harel and Engel, 2012; Collini et al, 2010; Panitchayangkoon et al, 2010; Engel et al, 2007; Hildner et al, 2013). The potential advantages of coherent quantum dynamics in energy transfer include, *inter alia*; robustness against disorder, enhanced rates via interference (constructive and negative), quantum thermal ratcheting, and lower driving forces for directed transport (Ishizaki and Fleming, 2012; Hildner et al, 2013; Mohseni et al, 2008; Plenio and Huelga, 2008; Caruso et al, 2009; Huelga and Plenio, 2013; Chin et al, 2013; Fassioli et al, 2014; Lambert et al, 2012). Although direct, unambiguous evidence for the beneficial role of quantum effects in photosynthesis remains elusive, an experimental example of how transient coherence can be crucial in organic photophysics has recently been presented by G  linas et al (2014), where ultrafast (< 80 fs) electron-hole separation at a bulk heterojunction only occurred when well-ordered acceptor phases which support delocalised electron states were present. This ultrafast, coherent electron dynamics leads to sufficiently rapid charge separation which can overcome the strong Coulomb binding energy that otherwise traps charges close to the heterojunction interface and drastically reduces their internal quantum efficiency (G  linas et al, 2014). As has been understood for a long time, the structure and motion of the protein environment is fundamentally important for understanding how quantum dynamics might impact on EET efficiency, and a nascent interdisciplinary field, known as quantum biology, has subsequently begun to

explore the surprising stability and role of quantum coherence in these structures. Acquiring a better understanding of these properties could provide interesting insights for other emerging types of quantum technology (Lambert et al, 2012; Huelga and Plenio, 2013; Anna et al, 2014; Scholes et al, 2011).

Though PPCs are ubiquitous in photosynthetic organisms, much of the attention related to quantum dynamics has focussed on the Fenna-Matthews-Olson (FMO) complex of green sulphur bacteria (GSB) (Van Amerongen et al, 2000; Blankenship, 2002). This structure facilitates the transport of electronic (excitation) energy from the antenna super-complexes to the reaction centres (RCs). Engel et al (2007) employed ultrafast femtosecond non-linear optical experiments to observe long-lasting (ps) quantum beating between electronic excited states in a four-wave-mixing response of an ensemble of FMO proteins. Although it is only found in GSB, the FMO complex carries out a typical and important function that is found in all the light reactions: namely, EET (Van Amerongen et al, 2000; Blankenship, 2002). The EET dynamics are driven by the dipole-dipole coupling of the pigments' optically excited states and the presence of a spatial energy gradient of local pigment excitation energies (Van Amerongen et al, 2000; Blankenship, 2002; Adolphs and Renger, 2006; Müh et al, 2007). The latter promotes irreversible energy relaxation that causes excitations to move to a specific spatially localised site or complex. It has been demonstrated that the FMO protein has evolved to drive the excitation close to the RC where this energy initiates the primary photochemical event of photosynthesis; charge separation (Wen et al, 2009).

As is now well-established, efficient EET dynamics depend on several key electronic parameters which are *controlled* by the pigment-protein structure; namely, (a) the local pigment optical transition energies (site energies), (b) the couplings between particular pigment optical transitions (excitonic couplings), and (c) the dynamical spectral density of protein fluctuations, which describes the strength and timescale of site energy modulations caused by the dynamic, thermal motion of the pigments' molecular surroundings and internal structure. This latter property provides the dissipative element required to drive relaxation along the excitation energy gradient. Many previous theories of EET in FMO have employed models which incorporate these parameters to varying extents, al-

lowing the prediction of kinetic and optical responses during EET. However, the necessity to include quantum coherence in the EET description introduces the need for new and sophisticated real-time simulations of density matrix evolution, higher precision prediction of key molecular parameters from *ab initio* techniques, and a reappraisal of the electronic and protein structure in terms of its capacity to host and coordinate quantum effects (Lambert et al, 2012; Huelga and Plenio, 2013; Anna et al, 2014; Scholes et al, 2011).

In this paper, we aim to provide an atomistic survey of the properties of the FMO protein, exploring the conformational motion and ensemble of its realisable structures using recently developed tools for analysing very large molecular systems. In our focus at all times will be the consequences of the multiscale, hierarchical structural relationships our simulations reveal, which we suggest facilitate coherent EET and aid interpretation of the experimental data. In this latter respect, a key result we shall present is the reduced disorder of the internal pigment arrangements from realisation to realisation. We suggest this property may act to suppress the inhomogeneous dephasing between excited states. This quality of the system is made possible by the highly coordinated motion of a hierarchy of structural elements in the FMO. This might offer some support for correlated-disorder theories that have attempted to explain the much weaker than expected effect of the substantial inhomogeneity observed in the ground state absorption of FMO on the longevity of inter-excited state coherences (Fidler et al, 2011). Moreover, we also speculate that the correlations of structural elements within *each* realisation might act to stabilise the static, average Hamiltonian structure of each configuration against inevitable deformations, helping the ensemble to perform efficient EET by ensuring that most of the members have functional electronic structure (see Section 2.3).

The structure of this paper is as follows: The key role of the protein structure in tuning (a)–(c), and how this affects quantum mechanical EET will be developed in Section 2. We will then present our methods for structural simulation and gathered results, which allowed us to visualise the conformational motion, spatial correlations, and a hierarchy of structural and dynamical relationships found in the FMO complex. Importantly, all of these observations appear to dramatically stabilise the internal co-ordination of the ‘working parts’ of this molecular wire. These are then analysed in

molecular detail in Section 5, after which we discuss a number of general organisational principles that act to stabilise EET in the presence of the slow protein motion that gives rise to conformational disorder. The possible relevance of the structure and correlated conformational motion for quantum dynamics and ultrafast optical experiments is highlighted, and we conclude with a few speculations on other types of quantum effect that might enable the *complete trimer structure* of FMO to avoid failures in EET transmission.

2 Pigment-protein crystal structure and an overview of its role in EET

The structure of the FMO complex is shown in Fig. 1. The FMO complex possesses a trimer quaternary structure in which each monomer assumes a clam-like architecture (Orengo et al, 1997) that sequesters seven pigments (Tronrud et al, 1986). The orientation of the FMO complex in the membrane positions an eighth pigment, which is located on the outside of each FMO monomer, near the baseplate (which is connected to the much larger light harvesting antenna complex known as the chlorosome) (Wen et al, 2009). The main low-energy optical absorption transition undergone by Bacteriochlorophyll a (Bchl) is the Q_y transition, which has an associated transition dipole moment that lies along the y-axis of the pigment (see Bchl 1 in Figure 1). The localised excited state created by either optical absorption or resonant energy transfer from another complex forms the basis of energy movement through the complex. This transport is thought to connect excitations accepted from the antenna complexes at Bchls 6 or 1 (from the baseplate or, possibly, Bchl 8) and move them to Bchl 3 (Wen et al, 2009; Adolphs and Renger, 2006; Müh et al, 2007).

2.1 Site energies

The effect of the protein on the site energies of the pigments has been previously investigated analytically using the static crystal structure (Adolphs and Renger, 2006; Müh et al, 2007; Adolphs et al, 2008; Gao et al, 2013). The protein environment was found to modify the site energy of the pigments in a number of ways. Hydrogen bonds formed between the protein and the pigments are found always

to red shift the site energy, while the electrostatic dipole moment of the α -helices are found to affect the transition density in a manner that depends on the relative orientation of the pigments in the protein matrix. For example, Bchl 3 displays a site energy shift of -103 cm^{-1} due to a hydrogen bond with Tyr 15 and -145 cm^{-1} due to the electrostatic potential of α -helix 7 (Adolphs et al, 2008). Similar effects of the protein on the local pigment Q_y site energies have also recently been found in fully-quantum mechanical simulations by Cole et al (2013). Interestingly, QM/MM calculations have shown that Bchl 3 exhibits the highest energy in the absence of the environment (Gao et al, 2013). This finding completely contradicts experimental observations, which have shown it to behave as an energy sink. Therefore, the presence of the protein determines the energy landscape of the pigments to create an energy funnel that relaxes excitation energy irreversibly toward the reaction centre (Wen et al, 2009; Adolphs and Renger, 2006; Müh et al, 2007). Maintaining this energy funnel at ambient temperatures, thereby avoiding the the formation of new, inefficient local minima in ‘poor’ conformations, appears to be enforced by a seemingly unique hierarchal structure which we shall describe.

2.2 Excitonic couplings

The spatial co-ordination of the pigments on the 10 –100 nm scale of typical PPCs is of crucial importance, as the radiative lifetime of a similar concentration of Chlorophyll-based pigments in solution would be far too short (due to concentration quenching) to transfer energy effectively (Yuen et al, 1980). The excitonic, or resonance energy transfer, coupling that occurs between pigments results from the Coulombic interaction between their transition dipole moments (Van Amerongen et al, 2000; Adolphs and Renger, 2006). In the absence of environmental interactions, this leads to the coherent (wave-like) spreading of an excitation over the pigments. Quantum mechanically, this can be understood as the formation of new excited states of the system which are coherently delocalised over the pigments (Van Amerongen et al, 2000; Adolphs and Renger, 2006). The strengths of these couplings are dependent on the mutual orientation and separation of the molecular transition dipoles. The degree of delocalisation of the excitonic states is a sensitive function of the strength of dipole-

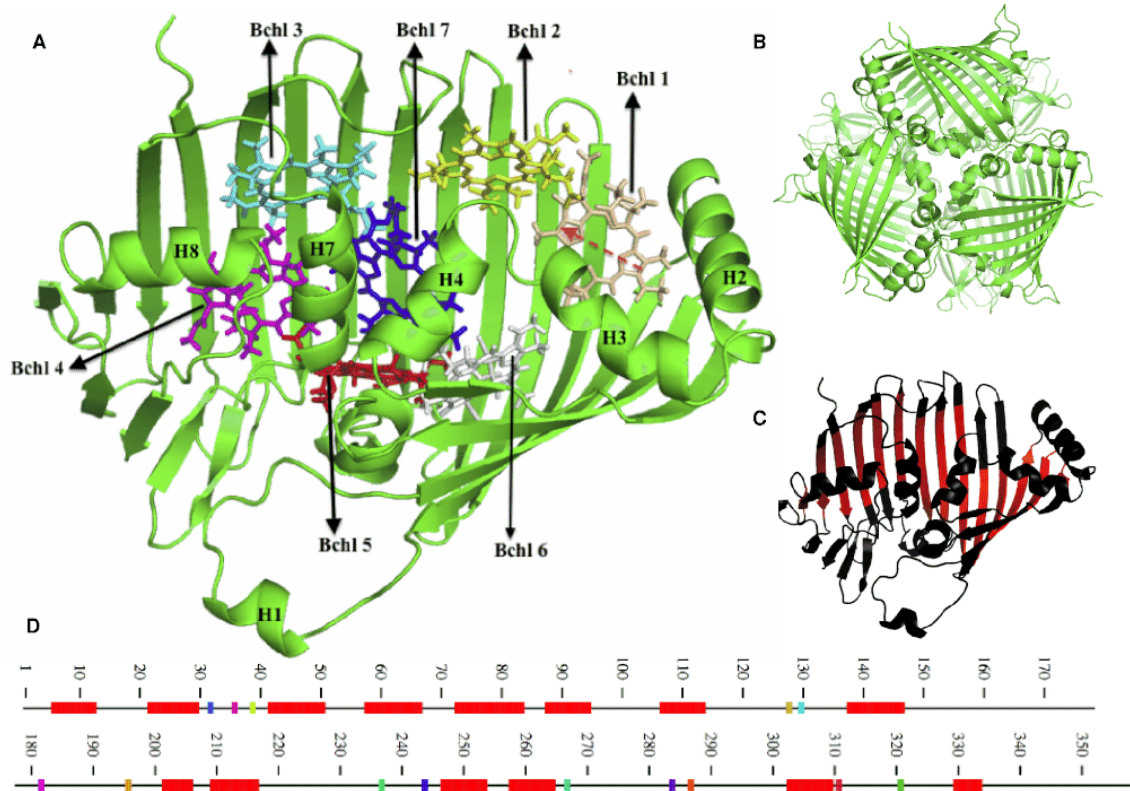


Fig. 1 (A) Monomer 1 of the FMO complex with the Bchl 1 to 7 and α -helices 1-5, 7, and 8 labeled. Bchl 1 has been used to illustrate the Q_y axis, which lies along the line formed by connecting the nitrogen atoms of pyrrole I and pyrrole III (red arrow). (B) The orientation of each monomer in the full trimer structure. The RCs identified by FIRST using $H_{cut} = -4.6$ kcal/mol are shown in red and flexible regions in black. These regions have been visualised on the monomer structure (C) and along the primary sequence (D).

dipole couplings and the differences in site energies of the pigments involved, such that coupling strengths significantly greater than the energy differences leads to fully delocalised excitons. In the FMO complex, the crystal structure ensures that the most significant couplings are those between nearest-neighbour pigments, creating coherently delocalised excitations. Due to the typically larger site energy differences between neighbouring pigments (compared to the excitonic couplings), this leads to excitations coherently delocalised over 1 – 3 pigments. These new ‘good’ quantum states, usually referred to as excitons, have modified optical properties and transition energies, the tuning of which could also be used to lend directionality to energetic relaxation dynamics. In 2D Fourier transform spectroscopies, broadband laser pulses are able to excite superpositions of these excitons.

The coherent optical interference is manifested as the quantum beats (discussed in the introduction) and the ps decay times are thought to indicate sustained quantum coherence in the EET process. However, if these effects are important for function, the FMO structure must be robust against any major variations in the relative pattern of the pigments' positions and orientations. Indeed, we shall show that the excitonic couplings, treated in the point-dipole approximation, are extremely robust ($< 5\%$) with respect to the allowed conformal variations supported by the FMO structure.

2.3 Dissipative protein fluctuations

The formation of an energetic ladder of delocalised excitations does not in itself lead to efficient EET, as a source of dissipation and dephasing is also required to force relaxation of the excitons (Van Amerongen et al, 2000; Adolphs and Renger, 2006; Plenio and Huelga, 2008; Caruso et al, 2009; Mohseni et al, 2008). This is provided by the excitable vibrational fluctuations of the pigments, protein and solvent environments which couple to the presence of locally excited electronic states, which is typically described using linear displaced oscillator models. These dynamical local interactions compete with the formation of delocalised states and cause dephasing between any prepared superposition of them. The strength and typical timescales of these fluctuations determines the qualitative character of the EET process. The kinetics fall within two extremes, namely; spatially localised, incoherent rate kinetics (Förster theory) and kinetics in the space of delocalised states (secular Redfield theory). This interpolation of behaviour is associated with strong and weak coupling relative to the strength of excitonic couplings, respectively. In both extreme cases, the existence of *inter-exciton* coherence does not impact on the EET kinetics. It is increasingly appreciated that many PPCs have roughly balanced excitonic and dynamical environmental interactions. This regime, where coherences may play a significant, albeit transient, role in EET, is less well understood. Exploration of open quantum dynamics in the “intermediate” regime, particularly with highly structured environmental spectra, is currently an area of intense activity (Christensson et al, 2012; Kreisbeck and Kramer, 2012; Tiwari et al, 2013; Chin et al, 2013; O'Reilly and Olaya-Castro, 2014; O'Reilly et al, 2012).

The environmental interactions that influence EET dynamically are fast, intra- and inter-molecular vibrations (10–1000s fs periods), typically of frequencies close to the energy differences between excitons. This is necessary for energy relaxation, as the phonons must be capable of absorbing these energies for the excitons to effectively relax. Classical molecular dynamics (MD) has been used to simulate atomistic motion on a ns timescale, but cannot be used to investigate important optical properties of PPCs, such as the effect of motions on the excited state site energies. MD simulations have therefore been used in combination with quantum chemistry calculations to investigate the excited states of the system. For example, ZINDO/S calculations have been applied to MD-generated snapshots to study (a) the electronic-vibrational couplings in the Q_y states of the pigments and (b) the transition energy fluctuations that are required to compute the environmental spectral density. This coupled QM/MM method has been applied to the RC of *Rb. sphaeroides* (Jing et al, 2012) and the FMO complex of *C. tepidum* (Olbrich et al, 2011b).

Unfortunately, the MD trajectory was unable to demonstrate the effect of long timescale structural changes (static disorder) on the exciton dynamics. This resulted in spectral features that are narrower than observed experimentally, as very low frequency contributions to the fluctuation spectrum provide the main source of pure dephasing processes and inhomogeneous broadening (Kreisbeck and Kramer, 2012). Additionally, using a related methodology to that described above, it was concluded that there is no correlation in environment-induced site energy fluctuations (Olbrich et al, 2011a) on femto-to-nanosecond timescales. This seemingly rules out the proposal that quantum coherences could be protected by dynamical correlation of site-energy fluctuations (Wolynes, 2009). However, the MD methodology used to simulate structural changes in the environment could only extend over 10 ns. It follows that some important characteristics could lie hidden in the ms timescales of static disorder that occur in natural biological systems.

With the inability of MD to account for relatively slow conformational dynamics of the entire FMO complex, the calculation of transition energies and their couplings to intra- and inter-molecular motion of the sluggish protein environment is a challenging task. Nonetheless, some progress has been made in this area. Normal mode analysis (NMA) has been employed to overcome the sampling

efficiency limitation that is met when employing MD simulations. In particular, NMA has been used to study the intermolecular exciton-vibrational couplings, which subsequently allows information about the spectral density to be obtained (Renger et al, 2012). However, although the low-frequency portion of the spectral density can be computed directly, there is no information about which of the normal modes are the most functionally, and therefore biologically, relevant (Ma, 2005).

In this study, the slow, conformational dynamics of the FMO complex will be simulated using the FRODA software, which takes as input geometric constraints that are calculated by the FIRST software (Wells et al, 2005) based on the experimentally derived structural properties of the protein. FRODA has been widely used, for example, in studying the impact of intracellular flexibility upon the conductance and gating of the 5-HT₃ and nictotinic acetylcholine receptor ion channels (Kozuska et al, 2014; Belfield et al, 2014), protein-protein docking involving multiple conformational changes (Jolley et al, 2006), elucidating cisplatin cross-linking in calmodulin (Li et al, 2012), and monitoring flexibility of myosin during the ATPase cycle (Sun et al, 2008). In contrast to force field based molecular mechanics simulations, the constraints-based Lagrangian dynamics that is implemented in FRODA effectively flattens the potential energy surface and substantially reduces the number of degrees of freedom to be explored. It has been shown that these simplifications actually allow FRODA to outperform MD in the sampling of transient pockets at protein-protein interfaces (Metz et al, 2011). Thus, the methods used here potentially allow the computationally efficient exploration of conformational space of the FMO complex, thus providing a tool to study the dynamical nature of the protein and to test theories concerning relationships between correlations and function, which complements information gained from the static crystal structure. Importantly, the resulting structural ensembles generally represent low frequency motions that are not necessarily small and thus go beyond the linear (harmonic) approximation, which is assumed when accounting for environmental dynamics by a spectral density, or normal mode analysis.

Therefore, by studying the concerted and correlated motions in the PPC, we hope to generate an all-atom picture of the biologically relevant motion present in the full FMO complex and thereby shed new light on the mechanisms that support EET and how long-lived quantum beats may appear

in ensemble experiments. However, we note here that the motion that we analyse potentially takes place on much longer timescales than EET, and is best thought of as a computational approximation to the configurational ensemble of complexes that is found in any experiment (which is the principle contribution to inhomogeneous broadening). Spatial correlations of site energies due to this motion, if present, could thus dramatically reduce the inhomogeneous broadening and might also help maintain the relative energy level structure, known to generate efficient EET, of the crystal structure in individual members of the ensemble (preventing, for example, the appearance of configurations where site 3 is not a sink, etc.). However, such effectively static correlations on the timescale of EET are not expected to lead to any dynamical protection of coherences.

3 Methods

Calculations were performed on the holo form of the trimeric 1.3 Å X-ray crystal structure of *Prosthecochloris Aestuarii* (PDB accession code 3EOJ). Full details of the structure preparation are described elsewhere (Cole et al, 2013). Briefly, hydrogen atoms were added to the structure using the MOLPROBITY software. A manual investigation of the structure resulted in the transfer of a proton from the ϵ to the δ nitrogen of His 6 (and the equivalent residues in monomers 2 and 3). The hydrogen atom of Tyr 9 and Tyr 338 sidechains was also rotated to form hydrogen bonds with Bchl 3 and 4, respectively. The AMBER11 software (Case et al, 2009) was employed for structural relaxation. The description of the protein was accomplished using the FF99SB force field, the water molecules using the TIP3P model, and the pigments by the force field developed by Ceccarelli et al (2003). Missing heavy atoms were added using the *leap* module of AMBER11. The structure was solvated and heated to 300 K over a period of 300 ps, followed by 1000 steps of conjugate gradients minimisation. Strong restraints (1000 kcal/mol/Å²) were applied to the heavy atoms of the protein and pigments throughout the equilibration procedure. A number of water molecules were found to play important roles in the pigment-protein hydrogen bonding network. Therefore, at the end of the equilibration procedure, the closest 600 water molecules to the pigments were retained to model the effects of specific pigment-water and protein-water hydrogen bonds.

Constrained geometric simulations were performed using the FIRST/FRODA software (version 6.2.1, downloaded from <http://flexweb.asu.edu>) (Wells et al, 2005; Wells, 2013). FRODA takes as input a decomposition of the protein into rigid and flexible clusters provided by the FIRST (Floppy Inclusions and Rigid Substructure Topography) software. FIRST identifies geometric constraints related to bond lengths and angles, hydrophobic interactions, and hydrogen bonds. Using the pebble algorithm, these constraints are used to determine flexible and rigid regions in the pigment-protein framework. The rigid regions form so-called rigid clusters that, as we shall show, display well-correlated motion due to their strong intra-connectivity. While the rigid clusters that form due to non-covalent interactions are the focus of the study, it should be noted that the porphyrin ring of the pigment molecules forms a rigid cluster due to the structure of the covalent bonds. The central Mg atom of each pigment was rigidly connected to the protein *via* the ligands identified in the X-ray crystal structure. Here, to generate rigid cluster decompositions, the hydrogen bond cut-off energy (E_{cut}) was set to -4.6 kcal/mol. The principal component subspaces spanned by FRODA simulations have been shown to be very robust with respect to the chosen value of E_{cut} (David and Jacobs, 2011).

FRODA (Framework Rigidity Optimized Dynamic Algorithm) efficiently simulates coarse-grained motion using the pre-determined geometric constraints output by FIRST. To generate a new conformation, the atom positions are perturbed in random directions by a magnitude of 0.1 \AA . This is followed by an iterative cycle that allows the constraints to be efficiently and accurately re-introduced by fitting the atomic positions back on to so-called “ghost templates” (which store the information relating to the previously identified constraints). It should be noted that, unlike MD, FRODA is not a dynamical technique and hence does not supply any characteristic time scales for the formation of individual structures. By significantly reducing the allowed degrees of freedom, FRODA is able to efficiently sample the relevant subspace of the total conformational search space. Such efficiency does come at the cost of being limited to a fixed constraints topology, generating an athermal ensemble and neglecting long-ranged electrostatic interactions and solvation (except for a small number of explicitly included water molecules). Yet even within these approximations, FRODA-generated atomic

fluctuations have been shown to agree with those identified in MD simulations and, critically, NMR experiments (Fulle et al, 2010), indicating that dynamics of the native state emerge naturally from a simple network of contacts.

FRODA was employed, in this study, to generate an ensemble of allowed structures of the FMO pigment-protein complex. We have performed 16 independent simulation runs and each has generated 80 000 distinct conformations. In order to gain a representation of the conformational ensemble, every 250th structure was stored for further analysis. Monomer 1 (as labeled in the PDB structure) was analysed in two simulations; a simulation in the absence of the other two monomers (the monomer simulation) and a simulation of the full trimeric structure (the trimer simulation). We have performed both monomeric and trimeric simulations, though all of the analysis that follows is with respect to the trimer simulation except where explicitly stated.

Within AMBER, the improved matrix facility of PTRAJ (Roe and Cheatham, 2013) was employed to generate the root-mean-square fluctuations (RMSF) of the C_α atoms of the stored conformations. The cross-correlation coefficient between pairs of atoms was also generated under this environment as follows:

$$C_{ij} = \frac{\langle \Delta r_i \cdot \Delta r_j \rangle}{\sqrt{\langle \Delta r_i \cdot \Delta r_i \rangle \langle \Delta r_j \cdot \Delta r_j \rangle}} \quad (1)$$

A score between two residues can range from +1 (correlated) to -1 (anti-correlated). This analysis allows the identification of long-range correlations present in the conformational ensemble, which may not be obvious from direct visualisation of the structure alone. Principal component analysis (PCA) was employed to extract biologically relevant motion using the Bio3d software package, which employs the R environment and has powerful statistical computing capabilities to generate the PCs (Grant et al, 2006). The lowest frequency modes identify the large amplitude concerted motions and are, therefore, intimately related to protein function and important in elucidating how the FMO complex is able to preserve quantum coherence in a noisy environment (Frank, 2012).

A fundamental process that occurs in PPCs, such as in the FMO complex, is resonance energy transfer. In a system of pigments with no orbital overlap, excitation energy transfer is mediated by Coulombic coupling between the transition densities of the chromophores. In the FMO complex, the

coupling interaction between pigments m and n (V_{mn}) has been shown to be well-reproduced by the point dipole approximation (Renger, 2008). This approximation uses the strength and direction of the transition dipole moment to estimate the distribution of excitonic couplings that are present in the ensemble of FRODA-generated structures according to:

$$V_{mn} = f \frac{\mu_{vac}^2}{R_{mn}^3} [\mathbf{e}_m \cdot \mathbf{e}_n - 3(\mathbf{e}_m \cdot \mathbf{e}_{mn})(\mathbf{e}_n \cdot \mathbf{e}_{mn})] \quad (2)$$

where \mathbf{e}_{mn} is a unit vector joining the Mg ions of pigments m and n and \mathbf{e}_m is a unit vector along the transition dipole moment of pigment m (defined by the line joining the N atom of pyrrole rings I and III). $\mu_{vac} = 6.1$ D is the transition dipole strength of the Bchl pigment in vacuum and its value has been experimentally-determined (Knox and Spring, 2003). The factor f describes the enhancement of the transition dipole moment in the protein environment, dielectric screening of the Coulombic interactions, and neglect of the dielectric cavities of the pigments. It has been shown, in comparisons with full solutions of the inhomogeneous Poisson equation, that $f = 0.8$ is a suitable scaling factor for the FMO complex. A more detailed discussion of the point dipole approximation can be found in Adolphs and Renger (2006).

4 Results

The trimeric structure of the FMO complex comprises 19,863 atoms. Therefore, to efficiently simulate motion on biologically relevant timescales we must employ some approximations to reduce the degrees of freedom of present in the system. To this end, Figure 1(c,d) shows a rigid cluster decomposition of the FMO complex using the FIRST software at a hydrogen bond energy cutoff (H_{cut}) of -4.6 kcal/mol. An unusual feature of the complex is the persistence of a large rigid cluster at low values of H_{cut} . This rigid cluster encompasses the majority of the clam shell (CS) architecture, which sequesters the optically-active pigments from the environment. The implications of the size and robustness of the CS rigid cluster will be discussed later. The internal structure of the complex, which mostly comprises the pigments and α -helices, is by comparison relatively flexible. Recall, it is widely accepted that the α -helices play a role in modulating the site energies of the pigments. Our

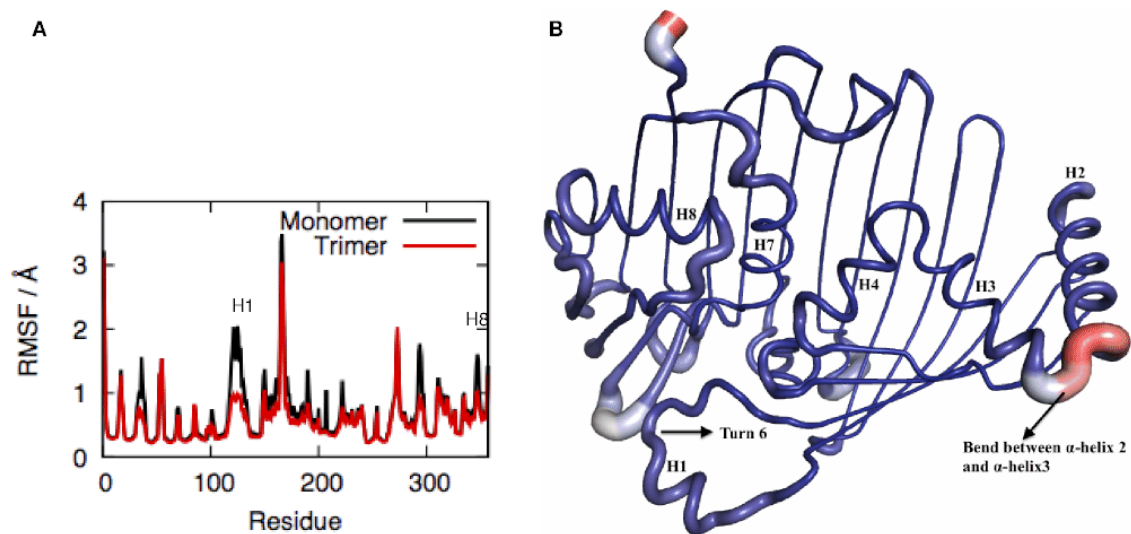


Fig. 2 (A) RMSF (\AA) per residue in monomer 1 from the monomer and the full trimer FRODA simulations. (B) The RMSF values from the trimer simulation have been displayed by colour (increasing from blue to red) and tube thickness.

choice of $H_{cut} = -4.6$ kcal/mol, therefore, allows us to efficiently explore the motion of the pigments and their environment within the approximation of a rigid CS structure. The FIRST rigid cluster analysis gives valuable information regarding the expected flexibility in the FMO complex. We have employed the FRODA software to further explore its allowed conformational space and elucidate the structural dynamics supported within these static results. FRODA took, as an input, the FIRST rigidity analysis at $H_{cut} = -4.6$ kcal/mol, which included 713 hydrogen bond and 236 hydrophobic non-bonded constraints. The RMSF calculated for each residue, averaged over the ensemble of output configurations, has been displayed in Figure 2. In the trimer simulation, the analysis reveals few regions with high conformational mobility – most residue fluctuations are lower than 1 \AA . One exception is the bend that connects α -helices 2 and 3 (residues close to 166). This is most likely due to the presence of three sequential glycine residues, which support the greatest backbone mobility. Note α -helices 2 and 3 are found to form direct non-bonded interactions with Bchls 1 and 2. A broad, mobile region is found from residues 120 to 130, which includes α -helix 1 and several residues either side. Another broad peak has been identified that encompasses α -helices 2 through 4. Small peaks that are caused by subtle motion of α -helices 7 and 8 can also be seen. Interestingly, the N-

and C- terminal ends of α -helix 8 and the N-terminal end of α -helix 7 are very close in sequence to several small β -sheets (that do not belong to the CS rigid cluster). Furthermore, these β -sheets are inter-connected via several non-bonded interactions and are more mobile due to their position on the end of the CS.

In the monomer simulation, we found α -helix 1 to have an RMSF that is more than twice that found in the trimer simulation. This is understandable because α -helix 1 forms direct contact with adjacent monomers in the full trimer structure. Less expectedly, the monomer also demonstrated much larger motion of α -helix 8 and turn 12 than in the trimer, and the bend between α -helices 2 and 3 was also found to increase slightly. Recall that α -helices, in particular α -helix 8, have been shown to strongly influence the optical site energies of the pigments (Müh et al, 2007). We therefore emphasise that the full trimeric structure should be used in dynamical studies of environmental fluctuations in the FMO complex.

Concerning the intramolecular atomic fluctuations of the pigments, we speculated that the average RMSF for each pigment may be related to the site energy disorder, and therefore the static inhomogeneous width and structure of the excitonic lineshapes in the linear absorption and linear and circular dichroism spectra (Van Amerongen et al, 2000; Adolphs and Renger, 2006; Shim et al, 2012; Cole et al, 2013). However, there is no significant difference in the RMSFs of pigments 1–7, averaged over the atoms of the porphyrin rings, which are small and range from 0.36 (Bchl 6) to 0.77 Å (Bchl 5). The contrast between these weak fluctuations and the much larger differences in site energy distributions identified by the MD simulation in Shim et al (2012), which generates a particularly broad spectral function on site 7, suggests that the site energy dispersion depends more strongly on interactions with the protein environment. The static influence of slow, conformational protein motion was not accessible to these techniques, and we now consider these in more detail.

We have already shown that the Bchl pigments of the FMO PPC, protected by a remarkably robust CS structure, display low conformational flexibility. This is particularly evident when comparison is made with the rigidity of structures that have previously been investigated with FRODA analysis (Wells et al, 2009). Following Wells et al (2009), rigidity was measured by counting the num-

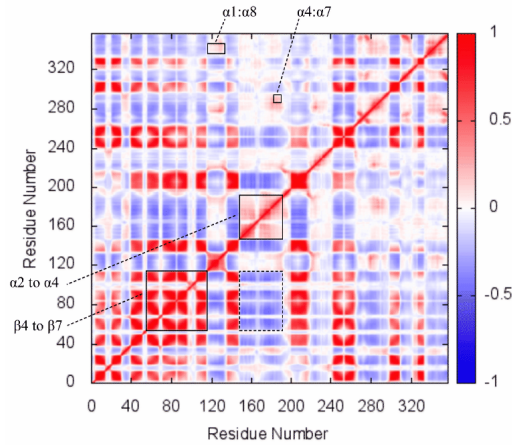


Fig. 3 The cross-correlation matrix for monomer 1 in the trimer simulation. Several noteworthy correlations between α -helices are highlighted. Also shown are β -sheets 4 through 7, which make up a large proportion of the CS structure – note also, that these structures are anti-correlated with the α -helices.

ber of C_α atoms that belong to the five strongest rigid clusters at $H_{cut} = -3.0$ kcal/mol in relation to the total number of C_α atoms (denoted f_5 , see Fig. 7 in Wells et al (2009)). Of the 51 proteins analysed in Wells et al (2009), only three proteins from a particular rigid protein family had a higher ratio than that revealed here for the FMO complex ($f_5 = 0.56$). Furthermore, the FMO complex is constructed from more than twice the number of residues as the most robust protein structures studied in Wells et al (2009).

A minimisation of flexibility (and thus thermal exploration of conformational states) of key structural elements (the CS and pigments) acts as a primary mechanism in the protection against the disorder of the Hamiltonian parameters which could disrupt the basic structure of the EET pathways. This is an expected requirement given the function of this pigment-protein complex. However, the seemingly excessive rigidity of the FMO protein suggests that this super structure may have been selected for purposes additional to simply holding the pigments in position. Indeed, we now show how this rigidity promotes and coordinates substantial correlations in the more flexible secondary elements of the structure. Computing the average cross-correlations between residue pairs in the ensemble of output structures from the FRODA simulation (Figure 3), we see that the residues belonging to the CS rigid cluster are extremely well-correlated over large distances. Interestingly,

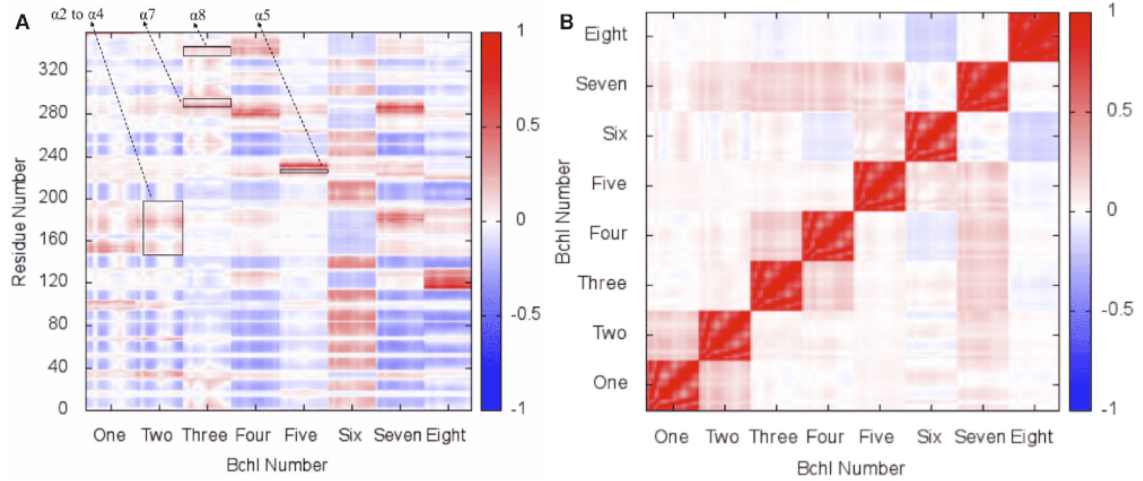


Fig. 4 (A) Correlations between the pigments and important secondary structure elements of the protein. (B) Correlations between the 8 Bchl pigments of the FMO complex.

these residues are also, generally, anti-correlated with the functional α -helices in the protein. It was also found that α -helices 4 and 7, as well as α -helices 2–4, displayed correlated motion with each other. The correlation between α -helices 2, 3, and 4 is particularly noteworthy given that they span a distance of roughly 24 Å and implies that long-range communication is present in the protein environment. β -strands 17, 19, and 20 are found to be flexible and reside at the end of the CS structure near α -helix 8. These strands are also anti-correlated with the CS rigid cluster, and display some correlation with the α -helices. This provides further evidence that the CS rigid cluster is involved in the maintenance of globally correlated conformational movement. Our results also demonstrate evidence for conformational correlations between pigments that are theoretically predicted to display strong excitonic coupling and form delocalised states (Adolphs and Renger, 2006; Adolphs et al, 2008) namely; between Bchl 1 and 2, Bchl 3 and 4, Bchl 5 and 6, and Bchl 7 with Bchl 1–5 (Figure 4(B)). Figure 4(A) shows the presence of correlations between the pigments and the protein environment. Firstly, with the exception of Bchl 6, the pigments display anti-correlated motion with the CS RC. Moreover, important correlations have been identified between secondary structure elements in the protein that, critically, modulate the pigment site energies. Bchls 1, 2, and 7 display correlated motion with α -helices 2 through 4. It is likely that this correlation gives rise to the observed correlated

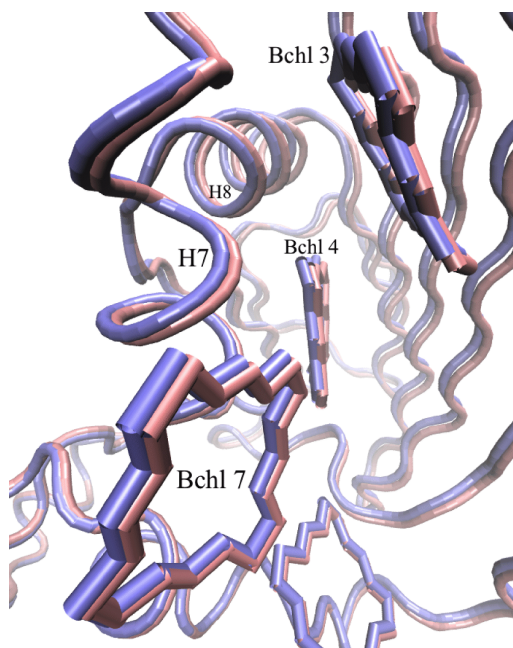


Fig. 5 The synchronised motion of α -helix 7 with pigments 3, 4, and 7 that is exemplified by the lowest frequency principal component.

motion with α -helix 7. Bchls 3, 4, and 7 also show strongly correlated motion with α -helices 7 and 8 (or residues adjacent in sequence).

Principal component analysis of the ensemble of structures from the FRODA simulation reveals the low frequency modes of the system, and allows us to visualise large-amplitude, concerted motions that may be relevant at long time scales. Here, the lowest frequency principal component provides additional evidence for the concerted movement of the functional α -helix 7 with pigments 3, 4 and 7 (Figure 5). The PCA has also identified low frequency motion for α -helices 1–4 and 8. It has been shown previously that the electrostatic dipoles of α -helices can significantly alter the local site energies of pigments (Müh et al, 2007; Adolphs et al, 2008; Cole et al, 2013). In particular, α -helices 7 and 8 are thought to be major contributors to the energy sink formed around pigments 3 and 4. Our observations of correlated (Figure 4(A)) and concerted (Figure 5) motion between pigments 3 and 4, and α -helices 7 and 8, make it highly plausible that spatial site energy correlations also exist. Such a mechanism would preserve the relative site energies of the pigments in the configurational ensemble and would potentially maintain Bchl 3 as the ultimate sink for EET in each conformational

Table 1 The inter-pigment excitonic couplings in trimer simulations of the FMO complex (cm^{-1}). The couplings identified by Adolphs and Renger (2006) (column 2) are compared with the mean excitonic couplings identified in the current analysis (column 3). The standard deviation for these couplings over the ensemble of output structures is displayed in column 4, where the coupled pigments displaying large σ have been highlighted in bold.

Pigments	Ref. Adolphs and Renger (2006)	This Work	σ
1-2	-98.2	-92.8	7.8
1-3	5.4	5.8	0.7
1-4	-5.9	-5.9	0.6
1-5	7.1	7.1	0.9
1-6	-15.2	-14.6	4.5
1-7	-13.5	-9.8	2.0
2-3	30.5	29.4	2.0
2-4	7.9	6.4	0.8
2-5	1.4	1.5	1.2
2-6	13.1	10.9	1.2
2-7	8.5	2.7	2.9
3-4	-55.7	-47.0	13.0
3-5	-1.8	-0.1	1.5
3-6	-9.5	-9.4	0.4
3-7	3.1	15.8	10.0
4-5	-65.7	-58.8	7.3
4-6	-18.2	-16.1	1.5
4-7	-58.2	-63.1	7.4
5-6	88.9	91.3	14.0
5-7	-3.4	-2.6	5.4
6-7	36.5	35.0	5.7

realisation of the complex. However, as mentioned previously, uncorrelated dynamical fluctuations on EET timescales (ps) are still required to drive fast energy transfer in the static energy landscape enforced by these correlated structures (Renger et al, 2012; Olbrich et al, 2011a; Rebentrost et al, 2009).

The formation of excitonic delocalised states is dependent on the extent of the dipole-dipole coupling interactions between the pigment molecules, where strong excitonic coupling gives rise to collective electronic excitations that have a high degree of delocalisation. This phenomenon depends strongly on the transition dipole moment which underlies the dipole-dipole coupling interaction V_{nm} between two pigments, which is, in principle, a sensitive function of the protein structure through its dependence on pigment-pigment separation and orientation. As discussed previously, an approximation of the coupling strength can be estimated from the transition dipole moments obtained from purely structure-based methods (Cole et al, 2013; Adolphs and Renger, 2006; Müh et al, 2007). Importantly, this analysis does not require costly quantum mechanical calculations. Table 1 shows the excitonic couplings and their standard deviations σ for monomer 1 from the trimer simulations. These values are compared with a previous study that used an identical method but was based only on the static crystal structure (Adolphs and Renger, 2006). The mean excitonic couplings of the ensemble of FRODA-generated structures, in general, agree with the calculations performed by Adolphs and Renger (2006). However, there are several noteworthy deviations between the previously calculated static couplings and the present data, which includes the effects of environmental fluctuations through the inclusion of structural flexibility. The largest deviation was found to be that between pigments 3 and 7. While the coupling calculated directly from the crystal structure was low (3.1 cm^{-1}), the current analysis has found that the averaged coupling over the conformational ensemble is higher, albeit with relatively large fluctuations ($15.8 \pm 10.0 \text{ cm}^{-1}$). A similar situation is identified for Bchls 3 and 4, as well as Bchls 5 and 6. The coupling between Bchls 3 and 4 displays the second largest deviation from the static coupling and also second highest σ . This link is also a part of the major EET pathway proposed for excitations entering the FMO complex via site 6 (Adolphs and Renger, 2006; Mohseni et al, 2008; Plenio and Huelga, 2008; Caruso et al, 2009). The small σ identified between Bchls 2 and 3 (2.7 cm^{-1}) is a significant finding. This shows that the excitonic coupling in an important link in one of the main EET pathways in the FMO complex (Adolphs and Renger, 2006; Mohseni et al, 2008; Plenio and Huelga, 2008; Caruso et al, 2009; Chin et al, 2010; Caruso et al, 2010; Rey et al, 2013) is preserved despite thermal motion in the en-

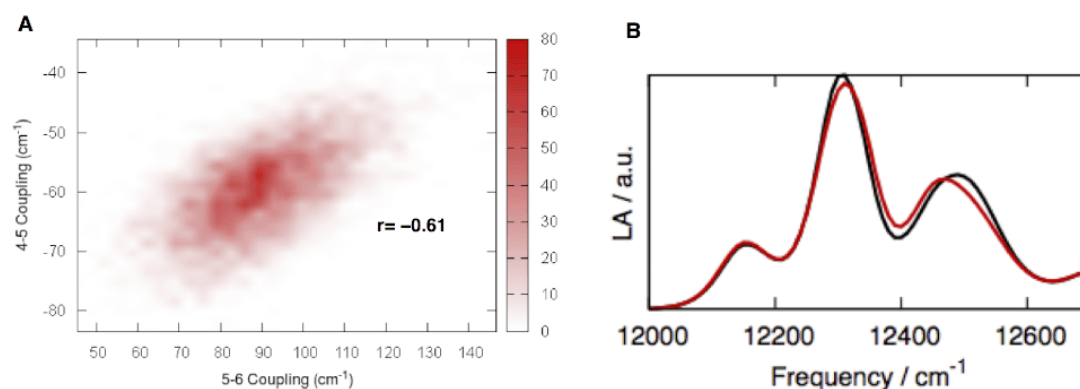


Fig. 6 (A) Heat map displaying correlations between the excitonic couplings found between Bchls 5 and 6 and Bchls 4 and 5. The negative Pearson's coefficient suggests that as the magnitude of coupling between Bchls 5 and 6 strengthens, the coupling between Bchls 4 and 5 diminishes. (B) Simulated linear absorption spectra for FMO using parameters described in the text. The plots show the spectrum computed using the mean values of the excitonic couplings (red line) and by averaging over the distribution of coupling strengths found by our FRODA-based analysis (black line). The high rigidity and conformational correlation between structures leads to very low spreads of couplings and negligible variation in the spectrum.

environment. The robust conservation of the coupling between these two pigments can be attributed to the conservation of their transition dipole orientation. This is predicted to be accomplished by a bend between β -strands 2 and 3. This bend is (a) able to span the distance between the two pigments due to its long length and (b) has the highest concentration of prolines in the amino acid sequence. Recall that proline residues can be employed to rigidify loops (Barzegar et al, 2009) due to their ability to reduce the conformational flexibility of the protein backbone. It may be that a conformationally restricted bend is a “just right” measure that allows the two pigments to remain connected while also allowing communication with other functional elements (α -helices, pigments, etc.). Although the principle findings with relation to the excitonic coupling is their small variability, correlations in this electronic property were also discovered. For example, Figure 6(A) shows the correlations in the excitonic coupling fluctuations identified between Bchls 5 and 6 and Bchls 4 and 5. Several other correlations were also found between Bchls 3–4 and 4–6 ($R=+0.62$) and between Bchls 4–7 and 3–7 ($R=-0.43$).

Finally, to investigate whether the excitonic structure of the FMO complex is robust with respect to the, apparently small, coupling fluctuations shown in Table 1, we have computed their impact on the linear absorption spectrum of the PPC at $T = 77$ K. The red line in Figure 6 is computed using the trimer pigment site energies taken from Adolphs and Renger (2006) and the mean excitonic couplings from Table 1. The theoretical treatment of the spectrum (Hamiltonian and secular Redfield theory), as well as all other parameters (spectral function, inhomogeneous disorder) are as described in Cole et al (2013). As can be seen in Figure 6, averaging the spectra over the distribution of coupling strengths, which are assumed to be independent, leads to negligible difference in the optical spectrum (black line). The spectra are instead dominated by homogeneous and site energy disorder. If our hypothesis that the strongly correlated spatial structures of each realisation of the complex also lead to correlated site energies in each ensemble member, then the excitonic structure and EET pathways will be highly conserved across the conformational ensemble. Such a mechanism for robust EET pathways and performance could be important for organisms such as GSB which make use of many thousands of FMO complexes in their functional photosynthetic apparatus.

5 Discussion

5.1 The Role of Motion in Protecting Efficient Energy Transport

The existence of persistent coherence in experimental ensemble signals relies heavily on suppressed disorder of the site energies of the coupled pigments in each complex and also between members of the experimental ensembles. As discussed above, these energies are significantly modified by the surrounding environment. In the current study, we present evidence for the (natural) selection of correlated conformational motion in the FMO complex. In light of previous work (Rebentrost et al, 2009), we speculate that this behaviour facilitates a means for correlating fluctuations in pigment site energies. This would allow realisations of the FMO protein that have significantly different band gaps between their manifold of optical excited states and the ground states to preserve the all-important energy differences between the states in the optically excitable manifold (Lee et al, 2007; Fidler et al,

2011). This design principle would naturally minimise the population of conformations that worsen or halt energy transfer (for example, by creating new energetic minima). By employing key structural correlations these disfunctional conformations are likely to have been selected against during the evolution of GSB. However, we draw the readers' attention to the fact that the significant correlations of atomic motion that we observe do not guarantee correlations of all electronic properties of the pigment network. While the impact of low frequency conformational changes in cases where electronic properties depend almost trivially on spatial coordination, such as dipolar excitonic coupling, is immediately clear, the effect on local site energies is much more complex. For instance, a recent MD study of Olbrich et al (2011a) found correlations of fast atomic motion that did not result in significant correlations of local site energy fluctuations (Olbrich et al, 2011a). Similarly, Renger et al (2012) found correlations of atomic motion via a normal mode analysis that resulted in a negligible impact on dynamical relaxation and decoherence. To fully establish the link between site energies and atomic and rigid cluster displacements will require further structure-based calculations on the wide range of configurations generated by our sampling methods, using techniques such as those recently reviewed by Renger and Müh (2013). In spite of this important caveat, the computational efficiency of the FRODA technique allows us to potentially explore relatively large and correlated motions that, as stated previously, go beyond the harmonic approximation. We expect this property would lead to some degree of correlation in the inhomogeneous distribution of excitonic energies in an ensemble of FMO complexes that were not evident in Olbrich et al (2011a) and Renger et al (2012). This is suggested by the significant correlated motion of pigments relative to the α -helices whose dipolar electric fields often dominate the local site energy shifts on each BChl. Other arguments for how these conformational motions may lead to correlated energy shifts are highlighted in the following detailed discussions of the hierarchy of motions that we report for FMO, though direct confirmation by further work is still required. We now discuss the details of these conformational structural relations and their correlations in more detail.

We hypothesise that the FMO PPC uses three main levels of structural organisation to reduce mobility and constrain the conformations of the protein to ones that support coherent EET (Fig-

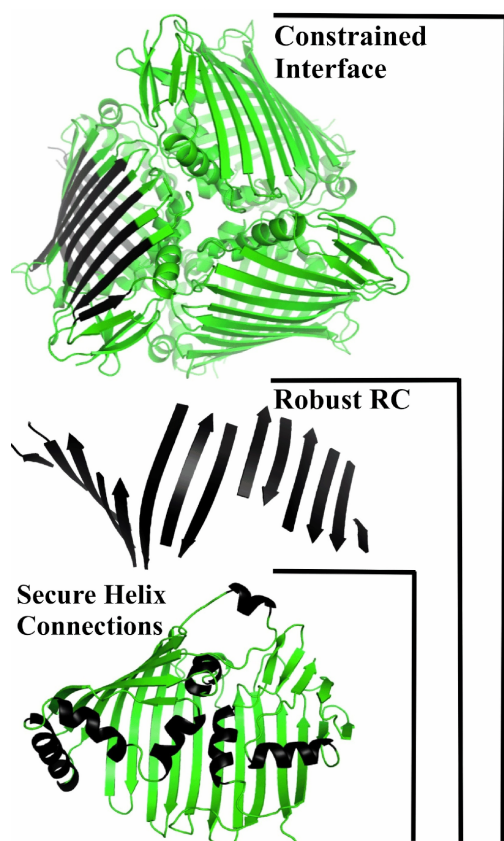


Fig. 7 Non-covalent interactions are employed to reduce the conformational fluctuations (uncertainty) in the motion of the FMO complex. As displayed above, these interactions can be grouped together into three main bands. Importantly, the observed correlations allow communication between the groups, an effect we have termed *trickle down structural organisation*.

ure 7). In what follows, we discuss the mechanisms by which specific non-bonded interactions preserve correlated motion between functional elements of the PPC in each of these three layers of structural organisation, thus safeguarding efficient EET.

5.2 Hydrophobic Interactions are Important for Inter-Pigment Correlations

EET is initiated from either Bchl 1 or 6, after which excitons relax towards Bchl 3 for entry to the RC (Brixner et al, 2005). The nearly degenerate Bchl 1 and 2 have been predicted to form a strongly delocalised pair of excitonic states, leading to a delocalisation-induced energy splitting and enhancement of EET in the pathway from sites 1 to 3 (Chin et al, 2010). Indeed, it has also been proposed

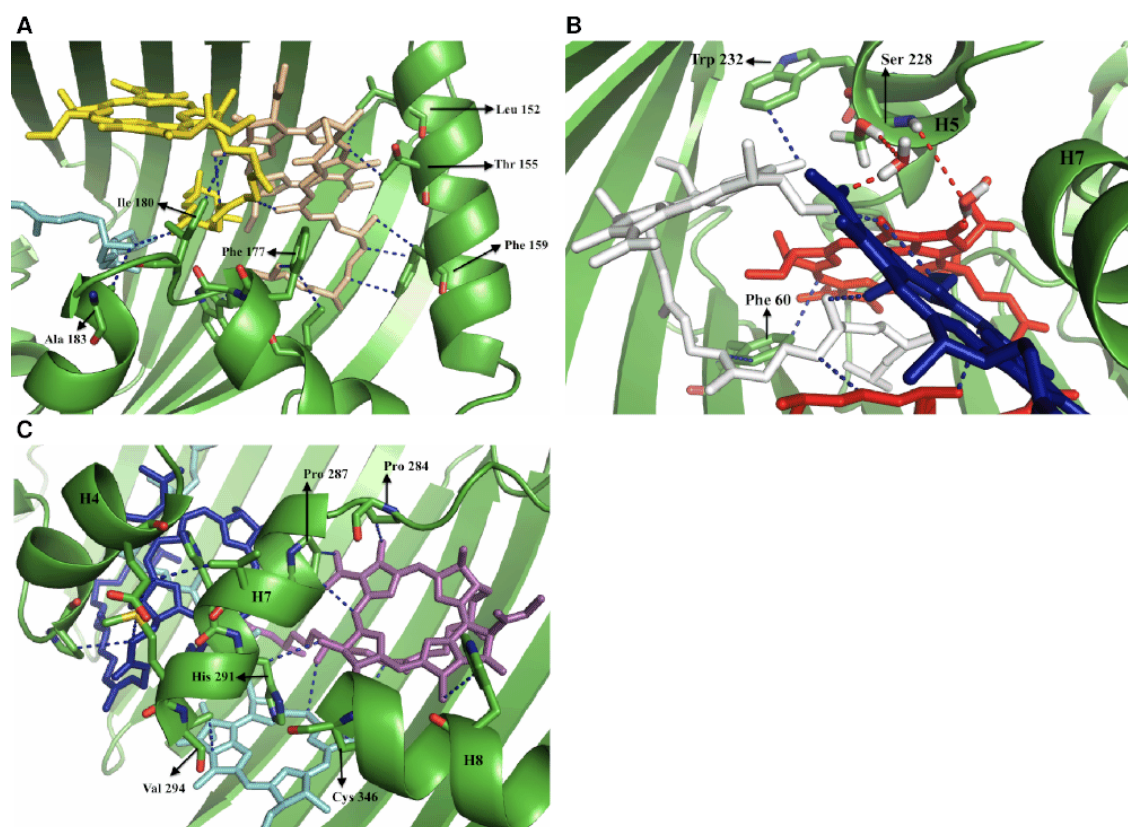


Fig. 8 (A) Extensive hydrophobic interactions between Bchl 1 (wheat) and α -helix 2 can be seen. Several hydrophobic interactions between Bchl 1 and Bchl 2 (yellow) are shown. Hydrophobic interactions are also found with residues in (Phe 177) and adjacent to (Ile 180) α -helix 3. (B) A hydrophobic interaction was identified between Bchl 6 (white) and turn nine residue Trp 232. The hydrophobic cluster that forms between Bchl 5 (red), 6, and 7 (blue) has been shown. A hydrogen bonding cluster was also identified, which involved Bchl 5, Bchl 7, Ser 228, and two water molecules. (C) Hydrophobic interactions are found to occur between Bchl 3 (cyan) and Bchl 4 (magenta). Bchl 3 and 4 are also found to interact with α -helix 7 (via Val 294 and His 291, respectively) and α -helix 8 (via Cys 346). Hydrophobic interactions were also identified between Bchl 4 and residues near in sequence or in α -helix seven. Note, an interaction was also identified with His 291, which coordinates Bchl 3. A hydrophobic cluster involving Bchl 7, α -helix 4, and α -helix 7 can also be seen.

that the excitonic splitting might be tuned to exploit a specific frequency of the environmental noise to achieve this enhancement, as a result of the so-called ‘phonon antenna’ mechanism (Rey et al, 2013). These quantum EET pathway dynamics require that the energy differences between sites 1 and 2 remain smaller than their excitonic coupling. The current analysis shows that these pigments

display correlated motion, and that their excitonic coupling varies negligibly across different conformational realisations. Although Bchl 1 and 2 interact directly with each other, their correlated movement is also maintained via interaction with nearby α -helices that display correlated motion with the pigments (namely, α -helices 2 through 4, see Figure 8(A)). Bchl 5 and 6 display correlated motion, and have been shown experimentally to facilitate coherent EET (Brixner et al, 2005). This is accomplished through non-covalent interactions between the two pigments (Figure 8(B)). Trp 232 is also well positioned to facilitate an indirect contact between Bchl 6 and turn 9 (which itself coordinates Bchl 5). The identified correlation between Bchl 5 and 7 relies on interactions with overlapping biological elements. Bchl 5 displays correlated motion with α -helix 5, which precedes turn 9 and, in turn, interacts with α -helix 7 and thereby provides an indirect connection between Bchl 5 and 7. These correlations are partially mediated by hydrogen bonds with water molecules.

It has been found that all the pigments (except Bchl 6, which will be discussed in the next section) are correlated with Bchl 7. From Bchls 5 and 6 the EET relaxes to Bchls 4 and 7. These two pigments are also found experimentally to participate in the same EET pathways (Brixner et al, 2005). Indeed, Bchl 3 and 4 interact directly with Bchl 7. However, correlated motion is presumably enhanced further by their mutual interactions with α -helices 7 and 8 (Figure 8(C)). Critically, these α -helices have previously been shown to affect the site energies of these pigments (Adolphs et al, 2008). The sensitivity of Bchl 7 to the motion of the other pigments suggests that it may play a unique role in the EET cascade. The importance of Bchl 7 has already been noted by Skochedopole and Mazziotti (2011) in connection with their research of quantum redundancy in the FMO complex. Therefore, although Bchl 7 is only present in one pathway, it may be a vital component for conserving important correlations in both EET pathways. This idea is further supported by the striking demonstration of Olaya-Castro and Fassioli (2011). Namely, optimised energy transfer through the FMO complex, as a function of temperature, is achieved by *maximal* excitonic correlations between site 7 and the rest of the pigments of the FMO complex. The correlations suggest that the pigment conformational motions remain in unison through *slow* correlated fluctuations managed by secondary structure elements.

We anticipate that correlated motions translate into spatially coordinated changes in the energy landscape that, on the timescale of EET, provide static maintenance of the key EET pathways.

5.3 The Robust CS RC

The user chosen H_{cut} was -4.6 kcal/mol. This allowed investigation of a network of interactions that (a) are stable (due to the extremely low cutoff) and therefore have the largest influence on the *in vivo* energy landscape and (b) give rise to geometric structures in the protein that are robust against time.

Connecting regions of a protein with particular roles assigned by Nature can reveal subtle design principles that are employed to improve a particular function. The uniqueness of the CS RC is a significant observation, and implies that it plays a critical role. In fact, such a large RC encompassing several secondary structure elements at low values of H_{cut} has not been seen in other systems (Wells et al, 2009). Indeed, the CS has an important structural role in the protein by sequestering the pigments and aiding the organization of the LHC by facilitating contacts with the baseplate, and perhaps promoting trimer aggregation in the periplasmic space between baseplates and RCs, which is another possible level of organisation which we do not deal with here. For example, the observation that 150-200 trimers contact the baseplate may demand tight packing that is facilitated by the CS RC (Blankenship, 2002). However, the extraordinarily robust RC is also efficient at transmitting structural information over distances of more than 50 \AA . In a previous work, the α -helices were found to have an unanticipated ability to modulate the site energies of all seven sequestered pigments (Müh et al, 2007). For example, the finding that Bchl 3 has the lowest site energy is a result of the dipoles of α -helices 7 and 8, which are able to shift the site energy by around -300 cm^{-1} . Natural selection facilitates the accumulation of residues that improve a protein's function (in this case, energy transfer). Therefore, it can be assumed that Nature has employed α -helices to manage the optical properties of the pigments and create an energetic funnel toward Bchl 3. The remarkable anti-correlation between the α -helices and the CS suggest the existence of a higher level of communication, which further encourages correlation between the spatially separated functional units. Since the CS

is well-correlated over large distances, it is an extremely well fitted candidate for mediating the long-range correlation of the functional α -helices. α -helix 7 is strongly anti-correlated with the CS RC and dictates the movement of Bchl 7. Recall, this pigment is correlated with many of the pigments in the system. α -helix 7 has bends either side that connect to the bottom and top of the CS. The reaction to the CS motion is therefore particularly strong. As a result, the motion of Bchl 6 (which is the only pigment that is correlated with the CS) seems to be anticorrelated with Bchls 4 and 7. The flow of information from the CS to α -helix 7, and on to Bchl 7 and the other pigments is a possible approach employed by the FMO complex to reduce uncertainty and maintain a robust electronic landscape for EET.

5.4 The Constrained Monomer Interface

Relative to the monomer simulation, the trimer displayed a decreased RMSF in several key regions of the FMO complex (Figure 2). One noteworthy example is the significantly decreased RMSF of α -helix 1 that occurs due to interactions with the adjacent monomer. It is important to note that α -helix 1 is (a) strongly anticorrelated with the motion of the CS in its own monomer and (b) strongly correlated with the motion of the α -helices and pigments in the adjacent monomer. Indeed, the motion of α -helix 1 of monomer 1 is found to be correlated with α -helices 2 through 4 ($C_{ij} \approx 0.5$), and Bchls 1 and 2 ($C_{ij} \approx 0.6$), of monomer 2. As the FMO complex displays rotational symmetry, α -helix 1 may act to communicate between the functional units of the trimer and thereby help constrain the conformations to (coherent) EET-competent ones. With the above observations in mind, it is predicted that the trimeric structure adds a further level of organization that is exemplified by the interactions of α -helix 1.

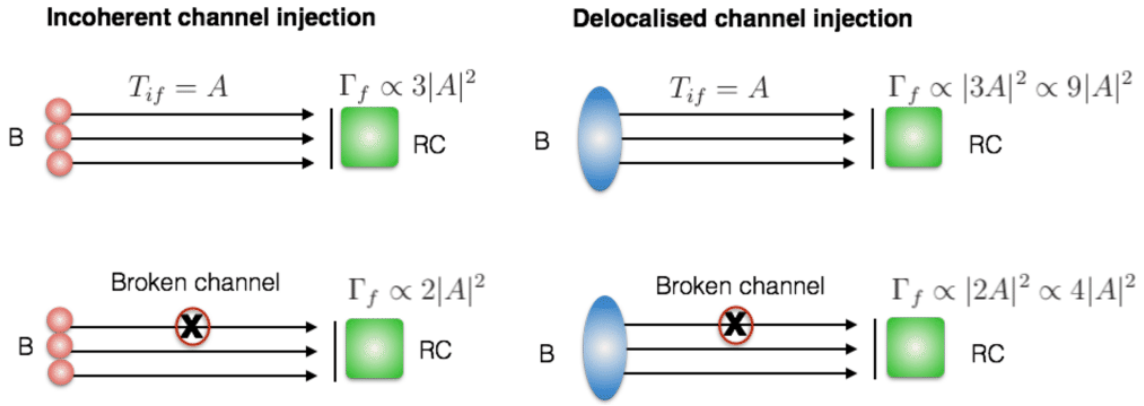


Fig. 9 A trimeric structure viewed as an ensemble of either classical or quantum wires. Quantum interference in the latter case can greatly increase average EET speeds over the classical wires, even in the case of a broken channel.

5.5 Trimer unit as a structure for maintaining energy throughput rates in the presence of functional failure

As a further and final speculation on the relevance of strong correlations in PPCs, we note that the use of a trimer structure – aside from considerations of supra-molecular stability – may also convey a degree of fault tolerance against failure of any of the individual monomers of the trimer, which could potentially be enhanced by quantum effects. Consider the situations depicted in Figure 9, which shows an abstracted picture of the process of EET through the FMO to the RC. Following the notion of the FMO monomers as molecular wires, we associate with each line (representing a monomer) a transmission probability for propagating an exciton to the RC. In a classical kinetics, and assuming that each monomer has, on average, the same transmission probability, is independent of the others and is equally likely to receive the excitation from the baseplate, a broken channel (due to disorder, mutation, etc.) would reduce the overall transmission probability for the trimer by 1/3 (Figure 9). Of course if the transmission were effected by a single monomer, a failure would be total: the trimeric structure therefore generally helps protect against this (not in itself a new observation). Under conditions of continuous reception of excitations a failure would lead to an average rate of excitation delivery of 2/3 of the fully functional rate.

In contrast, many of the results we have obtained suggest that intra- and inter-monomer disorder is suppressed to the extent that one could at least imagine the situation depicted in Fig. 9 (right). This shows a quantum analogue of the transmission process, where now the transmission is described in terms of the quantum mechanical amplitudes (propagators) A for excitons to reach the RCs through each monomer. In this description, the initial condition plays a key role in the subsequent EET. If the excitation starts randomly (incoherently) on any of the monomers then the average transmission probability is the same as the classical case if we associate the square of the amplitudes to the classical transmission probability. However, if the initial condition is a coherent superposition over the three initial entry points to each monomer, the final amplitude of transmission (assuming each monomer couples to the same RC) would coherently sum to $A_{total} = \sqrt{3}A$, leading to a three-fold increase in transmission probability, $P = 3|A|^2$ (meaning the rate of energy throughput is on average three times faster). This is a well-known (constructive) interference effect for waves, and appears in a variety of quantum contexts involving systems of common ground states (such as absorption and emission of photons in coupled chromophores). If, in the quantum case, a channel is broken, the total amplitude is now $2A/\sqrt{3}$ leading to a probability of $P = 4/3|A|^2$. Comparing with the classical case ($P_{cl} = |A|^2$), we see that the partially broken quantum wire still propagates excitations to the RC faster than even the undamaged classical transmission system. Although the quantum case maintains a higher propagation speed and average energy throughput, we note that in both cases a failure of a channel results in an overall loss of 1/3 of the total number of excitations.

This last idea is – emphatically – not a mechanism that we believe to operate in the FMO complex *in vivo*: the homogeneous dephasing rates between monomers is likely to be too great, and the injection mechanism from the baseplate may be too slow and random to prepare coherent superpositions of initial states. Indeed, it is not even obvious that enhanced transmission rates for excitons is something that is necessary or biologically desirable, although the idea that this structure – or a modified version – could adjust between trimeric and independent monomer operation, perhaps in response to metabolic needs or overload (to slow or speed up transfer), is an interesting idea for artificial quantum devices. However, these ideas are simply inspired from our observations of

the extent to which the protein correlates intra- and inter-monomer structures, and the (extreme) possibility that this could reduce decoherence across the trimer to facilitate injection into an initially delocalised state. While it is unlikely that this inter-monomer coherence could be sustained during the entire EET process, some recent theoretical work has shown that the first 50% of an excitation injected into site 1 of a monomer can propagate within a picosecond to the RC under Hamiltonian (coherent) dynamics alone, and – as this fast part of the transfer retains its wave-like character in the presence of noise – such pathways could be interfered (Mohseni et al, 2008; Plenio and Huelga, 2008; Caruso et al, 2009; Chin et al, 2010; Caruso et al, 2010; Rey et al, 2013).

5.6 Conclusion and prospects

In conclusion, we would like to suggest a number of ideas inspired by the wealth of structural information that we have gathered. We have stressed that the structural rigidity, correlated conformational fluctuations and inter-monomer interactions appear to suggest that the internal (monomer and trimer) energetic structure of the excited states may be conserved across different realisations of the protein and have shown explicitly that excitonic couplings show strikingly low variance across the ensemble of structures. This is particularly important for explaining the observation of quantum beats in 2D optical experiments, as the measured inhomogeneity of the one-quantum coherences would be expected to completely wipe out the zero-quantum (inter-exciton) coherences within 10-100 fs if the same disorder acted independently on each excited state. This is not what is observed. Preservation of the relative energy differences of the excited states, or signals dominated by sub-ensembles of particularly ‘coherent’ conformations seem to be the only plausible explanation (Lee et al, 2007; Fidler et al, 2011; Chin et al, 2013). Our results support the former picture, although both ideas are compatible with each other and are likely to contribute. We note that correlated disorder has recently been observed in the FMO complex (Harel and Engel, 2012), although other novel microscopic mechanisms appear to be required to reproduce the homogeneous decoherence times observed for zero quantum coherences in FMO (Christensson et al, 2012; Kreisbeck and Kramer, 2012; Chin et al, 2013). Another possible explanation for the reduced effect of inhomogeneity has been suggested by

Christensson et al (2012), who suggest that the quantum beats seen in 2D experiments are actually of intra-molecular vibronic character and only suffer effects of (much weaker) inhomogeneity of the vibrational frequencies. Clearly, a substantial amount of further work which could profitably combine the present techniques and those which address the faster molecular motions are required to describe the rich, multilayer photophysics of this and other PPCs. Anticipating these studies, we speculate here that the robustness in electronic parameters is not achieved by simply having an extremely rigid structure, but also by extensive correlation of conformational motion. As is briefly mentioned below, it could be that this motion has been selected for as being fit for purpose, and we speculate that adapting or modulating *motion or correlations* may be a much more energetically efficient and rapid way of responding to internal (external) needs (influences) than costly and possibly hard to reverse structural changes.

On a more general level, we believe that this study illustrates the potential of the computationally very efficient rigidity analysis and constrained geometric dynamics to tackle biomolecular problems in which the identification of key functional or structural units (rigid clusters), and a detailed, mechanistic description of their interplay, cannot be determined from the static crystal structure alone. The present problem of FMO provides a good example of how these techniques can move us further toward the oft-stated goal of “quantum biology” to provide design principles for future light-harvesting devices and quantum technologies, doing so at the all-important level of supra molecular structure and motion. In this context, an exciting prospect is to consider mutated and altered structures within the framework here established, looking for structures with stronger conformational correlations, reduced overall motion of key residues and/or rigid clusters, or increased intermonomer interaction. Moreover, the feature of being able to access low frequency motion *and* conformational distributions has provided new insight into the hierarchy of relationships that may govern the successful and robust function of FMO as an EET device, as quantified by our PCA and correlation results. The important role of low frequency motion in catalysis and other functions, and its selection in evolution has recently become a subject of great interest (Marsh and Teichmann, 2014), particularly the idea that slow, functional motion is more strongly conserved than other fluctuations

and that ensembles of molecular configurations generated in simulation often show strong correlation with the distributions of related structures produced by evolution. These topics, for which the methods presented in this paper are ideally suited to study, will be the subject of forthcoming work.

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